EFFECT OF SOME GROWTH SUBSTANCES ON THREE FRESHWATER GREEN ALGAE

P.V. DEVI PRASAD*

SUMMARY. — The effects of five growth substances (indole, 3- acetic acid, indole, 3-butyric acid, 2- indole propionic acid, naphthalene acetic acid and gibberellic acid) on three freshwater green algae - *Scenedesmus obliquus, Ankistrodesmus falcatus* and *Chlorococcum* sp. - were studied. Growth of *S. obliquus* was highly stimulated by almost all the growth substances at 1 to 20 ppm concentrations. Highet concentrations (more than 5 ppm) of indole acetic acid and indole butyric acid were inhibitory to the growth of *A. falcatus*. Only indole acetic acid was stimulatory to the growth of *Chlorococcum* sp. When used together on *S. obliquus*, indole acetic and gibberellic acid have shown little interaction. Indole acetic acid and gibberellic acid promoted the formation of four celled colonies in *S. obliquus*. The activities of the enzymes peroxidase and a-amylase were stimulated by indole acetic acid, indole butyric acid and gibberellic acid while naphthalene acetic acid was slightly inhibitory to a-amylase activity in *S. obliquus*.

RÉSUMÉ. — L'action de cinq substances de croissance (acide 3 indole acétique, acide 3-indole butyrique, acide 2-indole propionique, acide naphtalène acétique, acide gibberellique) a été testée sur 3 algues vertes d'eau douce, *Scenedesmus obliquus, Ankitrodesmus falcatus, Chlorococcum* sp. La croissance de *S. obliquus* est fortement stimulée pour presque toutes les substances de croissance à des concentrations de 1 à 20 ppm. Des concentrations d'acide indole acétique et d'acide indole butyrique supérieures à 5 ppm inhibent la croissance de *A. falcatus*. Seul l'acide indole acétique stimule la croissance de *Chloroccocum* sp. Utilisés ensemble, l'acide indole acétique et l'acide gibberellique agissent faiblement sur *S. obliquus* mais favorisent la formation de colonies à quatre cellules. L'activité de la peroxidase et de l'a -amylase est stimulée par l'acide indole acétique, l'acide indole butyrique et l'acide gibbérellique tandis que l'acide naphtalène acétique inhibe légèrement l'activité de l'a -amylase chez *S. obliquus*.

* Department of Botany, University of the West Indies, Mona, Kingston 7 Jamaica (W.I.).

INTRODUCTION

Growth substances, mainly auxin like and gibberellin like substances are reported to occur in some algae and external applications of growth substances are reported to effect the growth and developmental processes of several algae (PROVASOLI and CARLUCCI, 1974). The processes that are reported to be affected by growth substances include the stimulation of growth stimulation of the development of reproductive structures, etc. (THIMANN and BETH, 1950; JOSE and CHOWDARY, 1978; PUISEUX-DAO, 1956). In higher plants, the activity of the enzyme peroxidase is usually enhanced by auxin treatment (THIMANN, 1969) and gibberellin treatment generally increases the activity of the enzyme a-amylase (CLEALAND, 1969).

Relatively few studies have been made on the role of growth substances in the growth and development of algae, when compared to higher plants. In the present work, the effect of four auxins and gibberellic acid on the growth of *Scenedesmus obliquus*, *Ankistrodesmus falcatus* and *Chlorococcum* sp., their combined effect on *S. obliquus*, and their effect on the colony development peroxidase activity and a-amylase activity in *S. obliquus* were studied.

MATERIALS AND METHODS

The algae *Scenedesmus obliquus*, *Ankistrodesmus falcatus* and *Chlorococcum* sp. were isolated from a freshwater reservoir and axenic cultures were obtained using standard phycological methods (cf. STEIN, 1973). The cultures were maintained at 22 ± 1°C and 12 h light and 12 h dark cycle at a light intensity of 2400 lux.

Five growth substances, viz. indole, 3- acetic acid (AA), indole, 3- butyric acid (IBA), 2- indole propionic acid (IPA), naphthalene acetic acid (NAA) and gibberellic acid (GA) (all from Sigma) were used at concentrations ranging from 1 to 20 ppm.

Experiments were carried out in 100 ml Erlenmeyer flasks with 40 ml medium (CHU-10) in each flask with appropriate concentration of the growth substance. Experimental conditions were same as for the maintenance of the cultures. Growth was measured after 5 and 10 days of growth as cell numbers as well as optical density of the total pigment extract at 665 nm. Cell counts were made with the help of an improved nebauyer haemacytometer. Pigments were extracted in 95 % acetone.

Colonies of *S. obliquus* on which the effect of the growth substances on the colony development was studied, were counted in a haemacytometer. Different types of colonies present were counted and expressed as percentage of the total colonies present.

Peroxidase activity was estimated according to GAHAGAN et al. (1969). The reaction mixture contained 0.1 ml of cell free extract in 0.1 M phosphate

buffer, 2 ml of 16 mM hydrogen peroxide and 2 ml of 100 mM pyrogallol. The activity of a-amylase was estimated by the method of KHAN and FAUST (1967). Protein was estimated according to LOWRY et al. (1959).

RESULTS

Growth : Both cell counts and the optical density of the pigment extract showed same trend. Hence only optical density results are presented. Tables 1 to 3 give the results obtained with growth experiments. Indole acetic acid had a positive effect on the growth of *S. obliquus* and *Chlorococcum* sp. while it had a negative effect on the growth of *A. falcatus*. Only the growth of *S. obliquus* was stimulated by IBA. High concentrations of IBA were toxic to *A. falcatus*. A modest increase in the growth of the algae was caused by IPA as compared to the control. *Chlorococcum* sp. and *A. falcatus* did not respond to NAA while the growth of *S. obliquus* was stimulated by the same auxin. Gibberellic acid gave similar results as NAA.

When used together, only the combination of the lowest concentration of the auxin and highest concentration of GA and the combination of highest concentration of IAA and lowest concentration of GA gave considerable increase in growth over control (Table 4) in *S. obliquus*.

Colony development is *S. obliquus* : Formation of four-celled colonies was promoted by GA while NAA treatment resulted in the formation of maximum two-celled colonies by 5th day. However, by the end of 10th day, IAA also promoted the formation of four-celled colonies along with GA, while NAA treatment was like control in having almost 50 % each of 2-celled and 4-celled colonies (Tables 5 and 6).

Enzymes : The activity of peroxidase was enhanced by IBA by about 50 % and by GA by about 30 % in *S. obliquus* (Table 7). The activity of the enzyme a-amylase was enhanced by IAA by about 40 % and by GA by about 60 %, while NAA reduced the activity by about 20 % (Table 7).

DISCUSSION

Of the three algae studied, the growth of *A. falcatus* was not considerably increased by any of the growth substances used. Moreover, high concentrations of IAA and IBA reduced the growth of this alga. However, the growth of the other two algae was stimulated by all the growth substances studied.

AHMAD and WINTER (1968) observed that IAA stimulated the growth of several blue green algae and some green algae including *Chlorella pyrenoidosa*, *Scenedesmus obliquus* and *Ankistrodesmus falcatus*. In their study, AHMAD and WINTER (1968) reported the stimulation of growth of the green algae at 10^{-3} M IAA and they did not find any inhibition at high concentration of the auxin. However, the present results are in contrast with AHMAD and

TABLE 1

Concentration (ppm)	Growth expressed in relation to control									
	IAA		IBA		IPA		NAA		GA	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
[illegible]	[illegible]	[illegible]	1?5.8	98±7.6	108±8.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]8	[illegible]	[illegible]	80±6.0	115±?8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]6.5
[illegible]	[illegible]	[illegible]	[illegible]	39±4.7	127±7.1	[illegible]	1?8±8.[illegible]	[illegible]	[illegible]	[illegible]3
[illegible]	?15.2	[illegible]±4.8	[illegible]	18±3.2	147±8.4	119±[illegible]	123±7.[illegible]	103±6.[illegible]	[illegible]	94±6.5
[illegible]	34±4.[illegible]	?1±3.[illegible]	1?±2[illegible]	dead	9?±6.1	114±7.3	121±7.[illegible]	103±6.[illegible]	96±[illegible]	88±5.9

TABLE 2:

Concentration (ppm)	Growth expressed in relation to control (control = 100)									
	IAA		BA		IPA		NAA		GA	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
[illegible]	112±6.[illegible]	[illegible]	81±5.7	84±6.0	100±6	102±6.3	101±5.9	103±6.5	104±7.3	101±6.8
[illegible]	116±6.3	1[illegible]±8.1	8?±7	99±6.[illegible]	[illegible]5	129±7.9	112±6.[illegible]	1[illegible]±6.2	??±5.4	11?±7.5
[illegible]	96±6.6	214±11.[illegible]	?7±6.9	82±6.[illegible]	86±6.8	130±9.9	88±6.8	110±7.5	91±6.5	??±7.5
[illegible]	80±6.[illegible]	[illegible]	[illegible]±7.5	67±[illegible].8	[illegible]	[illegible]	83±6.[illegible]	95±7.4	92±6.6	86±[illegible]
[illegible]	75±5.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]±5.1	[illegible]4±10.8	[illegible]	[illegible]	8?±6.6	76±[illegible]

TABLE 3

Concentration (ppm)	Growth expressed in relation to control (control = 100)									
	IAA		IBA		IPA		NAA		GA	
	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days	5 days	10 days
[illegible]	[illegible]8±[illegible]	[illegible]	[illegible]	110±8.[illegible]	[illegible]	[illegible]±9.[illegible]	103±5.9	198±[illegible].6	157±9.[illegible]	15?±8.9
[illegible]	1?1±7.[illegible]	[illegible]	[illegible]	108±6.9	10[illegible]	117±6.9	134±8.[illegible]	134±9.3	175±11.5	154±1[illegible].9
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	12[illegible]±9.8	137±8.5	100±6.5	224±11.2	79±[illegible].5	[illegible]56±1[illegible]
[illegible]	[illegible]	[illegible]±11.7	[illegible]	17[illegible]±8.[illegible]	11[illegible]	152±12.5	[illegible]2±6.[illegible]	206±15.2	214±1[illegible].5	[illegible]64±12.5
[illegible]	[illegible]	[illegible]±9.[illegible]	114±[illegible]	134±[illegible]	[illegible]	[illegible]	[illegible]7±7.9	[illegible]±12.5	125±8.6	10?±7.3

TABLE 4:

[illegible]	[illegible] ppm		10 ppm		5 ppm	
	5 days	10 days	5 days	10 days	5 days	10 days
5ppm	27±8.5	[illegible]±6.5	146±8.6	124±8.6	210±13.2	145±10.5
10ppm	25±10.5	[illegible]±6.9	[illegible]3±9.5	1[illegible]±7.9	123±9.5	145±10.5
15ppm	1?0±12.6	?5±9	1?7±8.9	110±9.3	125±8.6	105±11.9

Table 1. — Effect of five growth substances on the growth of *Ankistrodesmus falcatus*.
Table 2. — Effect of five growth substances on the growth of *Chlorococcum* sp.
Table 3. — Effect of five growth substances on the growth of *Scenedesmus obliquus*.
Table 4. — Effect of various combinations of IAA and GA on the growth of *S. obliquus*. (Growth expressed in relation to control, which is taken as 100).

TABLE 5

Concentration (ppm)	IAA		IBA		IPA		NAA		GA	
	2*	4	2	4	2	4	2	4	2	4
0	[illegible]	45±5.5								
1	47±[illegible]	51±5.1	47±4.2	53±4.2	88±7.5	11±7.5	90±8.8	10±8.8	50±4.7	50±4.7
	43±4.5	57±4.5	42±4.3	58±4.3	75±6.5	25±6.5	87±6.5	11±6.5	47±5.1	53±5.1
	54±5.2	43±5.2	55±4.8	45±4.8	68±7.1	3-+7.1	91±7.8	9±7.8	35±5.9	65±5.9
	57±4.9	42±4.9	62±5.5	37±5.5	58±4.7	40±4.7	86±9.3	12±9.3	27±7.5	73±7.5
	67+5.6	33+5.6	60±5.8	31±5.2	37±6.3	61±6.3	91±8.8	8±8.8	17±7.8	83±8.8

*= two-celled colonies; 4= four-celled colonies. Where the total does not add up to 100, the remaining are unicells.

TABLE 6

Concentration (ppm)	IAA		IBA		IPA		NAA		GA	
	2*	4	2	4	2	4	2	4	2	4
0	48±5.2	52±5.2								
1	54±4.5	46±4.5	58±6.1	42+6.1	32±7.1	66±7.1	61+5.8	39±5.8	35±6.5	65±6.5
	46±5.4	54±5.4	0±6.3	30±6.3	36+5.4	64±5.4	52±5.6	48±5.6	32±4.9	68±4.9
	30±4.1	70±4.	62±6.7	38±6.7	3 +5.6	65±5.6	47±4.5	53±4.5	28±6.2	72±6.2
	30±4.1	70±4.9	57±5.6	41±5.6	32±6.9	68±6.9	45±5.6	54±5.6	24±7.0	76±7.0
20	11+7.6	89±7.3	42±7.8	58±5.1	28±6.5	72±6.5	45±5.3	55±5.3	12±7.5	88±7.5

*2 = two-celled colonies, 4 = four-celled colonies. Where the total does not add up to 100, the remaining are unicells.

TABLE 7

Enzyme	Control	IAA		IBA		IPA		NAA		GA	
		5**	15	5	15	5	15	5	15	5	15
Peroxidase	0.09	0.095	0.10	0.110	0.135	0.095	0.105	0.095	0.110	0.095	0.115
a-amylase	0.05	0.07	0.065	0.055	0.060	0.050	0.050	0.050	0.040	0.080	0.070

* Activity of the enzyme is expressed per mg protein/min

** Concentration of the growth substance (ppm)

Table 5. — Effect of five growth substances on the colony formation in *Scenedesmus obliquus* after five days of growth.

Table 6. — Effect of five growth substances in the colony formation of *S. obliquus* after ten days of growth.

Table 7. — Effect of five growth substances on the activity* of the enzymes peroxidase and a-amylase in *S obliquus*.

WINTER (1968) as IAA at a low concentration of 1 ppm was slightly stimulatory to *A. falcatus* while concentrations above 5 ppm were inhibitory to *A. falcatus* even at 1 ppm. IBA in the present study was also inhibitory at even the lowest concentration to *Chlorococcum* sp. The growth of *S. obliquus* was significantly increased by almost all the growth substances. The present results do not agree with AHMAD and WINTER (1968) who concluded that IAA was stimulatory at higher concentrations only but agrees with CONRAD et al. (1959) who observed the stimulation at low concentrations and inhibition at higher concentrations. The differential response of *A. falcatus* to IAA as reported here and by AHMAD and WINTER (1968) could be due to the difference in the stratins used.

Gibberellic acid caused tubular elongation in *Ulva lactuca* at a concentration of 0.0a ppm (PROVASOLI, 1958). The growth of *Chlorella vulgaris, C. pyrenoidosa, S. obliquus* and *S. quadricauda* was also observed to be stimulated by GA (SAONO, 1964). The present results were similar to SAONO (1964). However, the increase in growth was considerably more in the present study when compared to the 20 % increase in by *S. obliquus* observed by SAONO (1964).

Experiments with various combinations of IAA and GA with *S. obliquus* do not allow to conclude positively whether these two acted antagonistically or synergitically. However, whenever these two were at same level, the growth was always less than that obtained with same concentrations of GA alone suggesting a possible antagonism between the two growth substances.

The species of the genus *Scenedesmus* tend to be polymorphic under different nutritional and environmental conditions (TRAINOR et al, 1976). Control of colony formation with the elimination of the unicellular stages in some strains of the genus was achieved by TRAINOR and SCHUBERT (1974) by using very dilute media. Present results suggest that growth substances, specially GA and IAA may also have a role in the colony formation as the population of four-celled colonies almost doubled over control.

The activity of the enzymes peroxydase and a-amylase was increased considerably by IAA, IBA and GA in the present study. In the higher plants, auxins are generally thought to be stimulatory for the peroxidase (THIMANN, 1969). Similarly, GA is considered to be stimulatory for the a-amylase (CLEALAND, 1969). However, GA was observed to inhibit peroxidase activity of the dwarf strain of Alaska pea (McCUNE and GALSTON, 1959) while GA increased the peroxidase activity in the endosperms of barley seeds where IAA failed to increase the same (HARMEY and MURRAY, 1968). In the present study, IAA. IPA and NRA did not effect peroxidase activity while IBA increased the same by about 50 % at 15 ppm in *S. obliquus*. This does not seem to have any relation to growth as growth at this concentration was not different from the control.

Activity of a-amylase was in general low. However, it is interesting that IAA enhanced the activity by about 40 %. The effect of GA, which increased the activity of the enzyme by about 60 %. appears to be similar to tits effect on higher plants.

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